

Control survival of mussels in laboratory toxicity tests

Chris Ingersoll, Ning Wang, Chris Barnhart, Dick Neves

USEPA Mussel Toxicity Testing Workshop
Chicago, IL, August 23 and 24, 2005



Overview

- Test acceptability requirements in E2455-05:
 - 24-h glochidia: 90% survival (must)
 - 96-h juvenile: 90% survival (must)
 - 28-d juvenile: 80% survival (should)
- Example data sets
 - Historic data cited in E2455-05
 - USGS-Columbia data
 - Culture of juvenile mussels in the laboratory



Guidance in ASTM E2455-05

- 9.2.4 “A dilution-water control must be included in the test and the survival and growth of test organisms in the dilution-water control **must meet test acceptability requirements** in order for the test to be considered acceptable.”
- 10.6.3 “Little is known about the survival, growth, and reproduction of naturally produced mussels once the juvenile mussels excyst from the host organisms.”

Example data sets

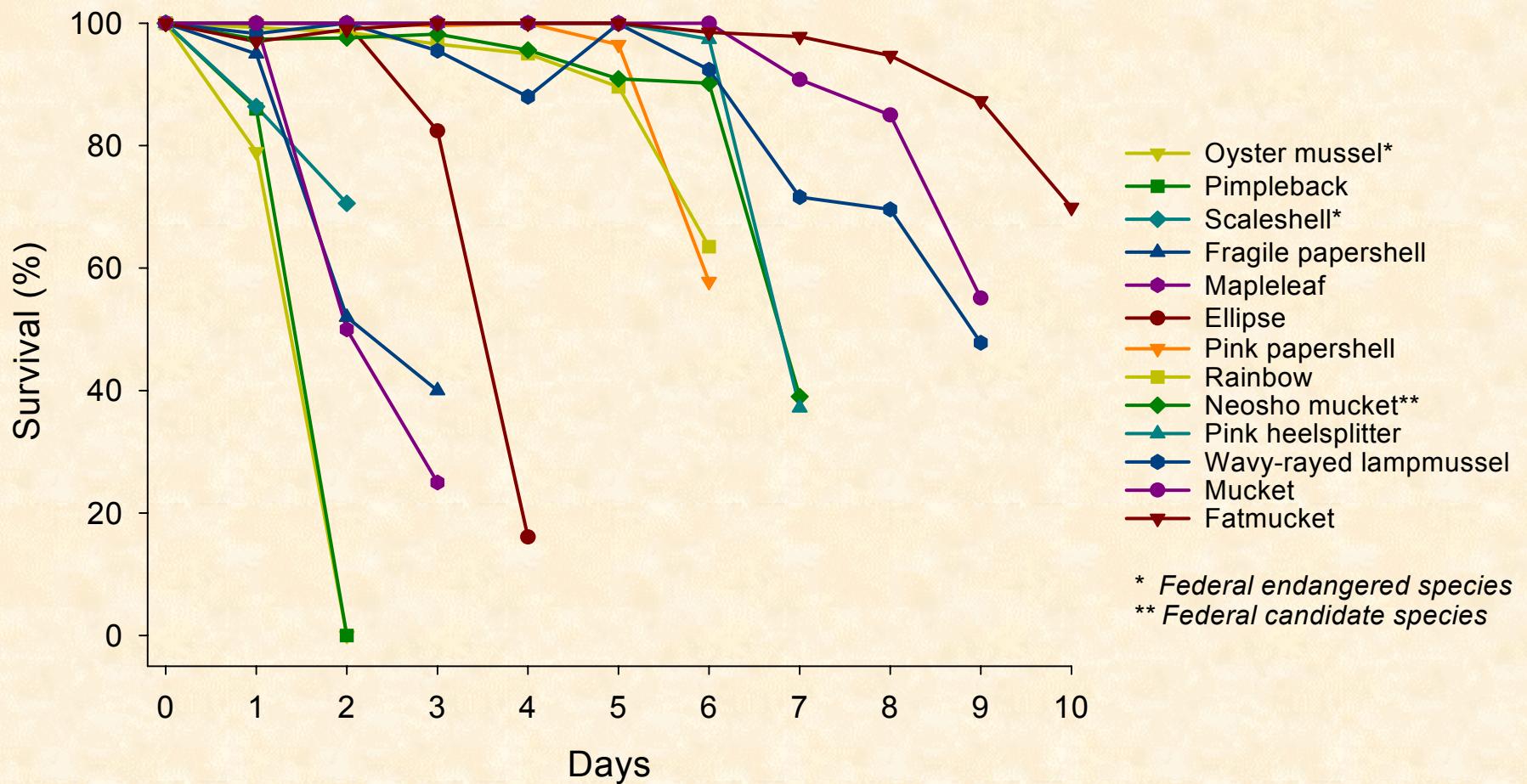
- Historic data cited in E2455-05:
 - Glochidia 48-h survival >90% in 4 of 9 studies (Table A1.1)
 - Glochidia 48-h survival >90% in 5 laboratory participating in inter-laboratory testing (Table 4)
 - Juvenile 96-h survival >90% in 13 if 14 studies (Table A1.4)
 - Juvenile 96-h survival >90% in 5 laboratory participating in inter-laboratory testing (Table 4)
 - Juvenile 21- to 28-d survival >88% in 4 studies (Table A1.4)

Table A.1.2. Survival time of glochidia after removal from female unionid mussels

Species	Temperature C	Duration of viability			Reference
			Day	(% survival)	
<i>Actinonaias ligamentina</i>	20	7 (>90);	8 (>75);	9 (>50)	USGS (2004)
<i>Actinonaias pectorosa</i>	10	13 (>75)			Zimmerman and Neves (2002)
	25	5 (>75)			Zimmerman and Neves (2002)
	20	>2 (>90)*B			Jacobson et al. (1997)
<i>Alasmidonta heterodon</i>	20	2 (>90);	2 (>75);	2 (>50)	USGS (2004)
<i>Anodonta anatina</i>	13	>3 (>90)			Huebner and Pynnonen (1992)
<i>Anodonta cataracta</i>	10	>14 (>90)			Jacobson (1990)
<i>Anodonta cygnea</i>	13	>3 (>90)			Huebner and Pynnonen (1992)
<i>Anodonta grandis</i>	10	>14 (>90)			Jacobson (1990)
<i>Elliptio complanata</i>	5	7 NR			Matterson (1948)

Data summarized for 34 species in Table A1.2
of ASTM E2455-05.

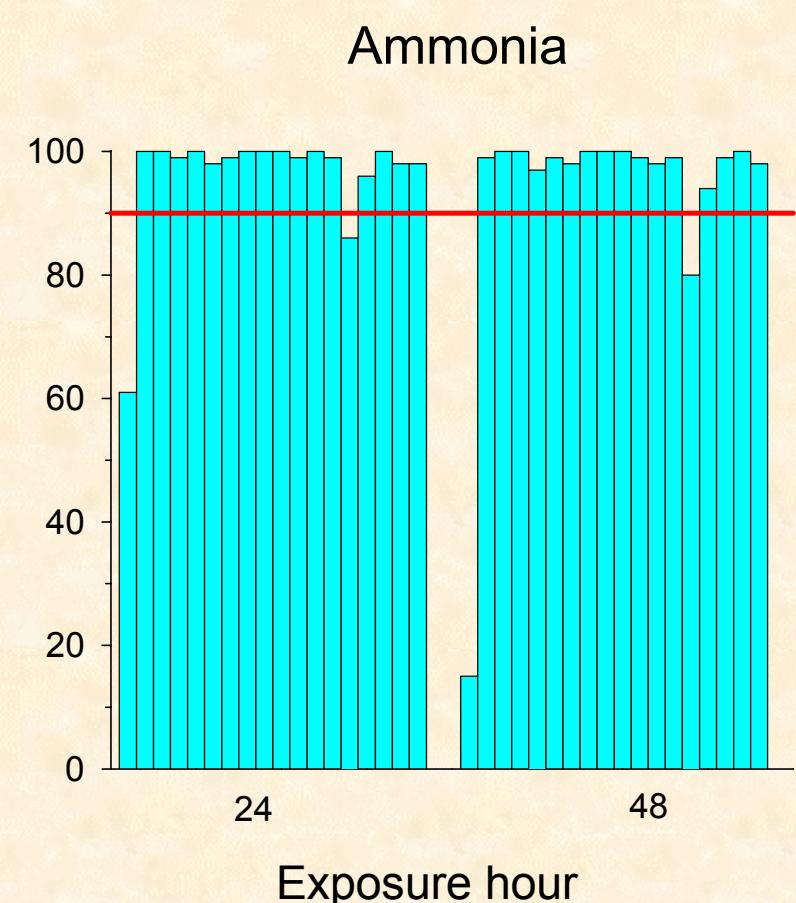
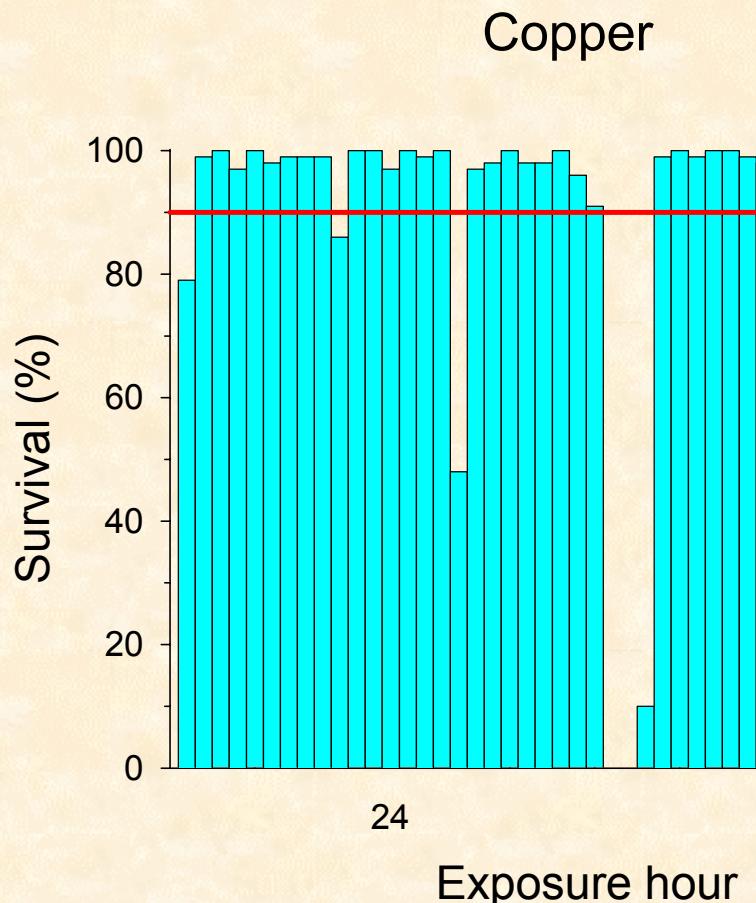
Survival time of glochidia after removal from female mussels



Survival time of glochidia held in different test water

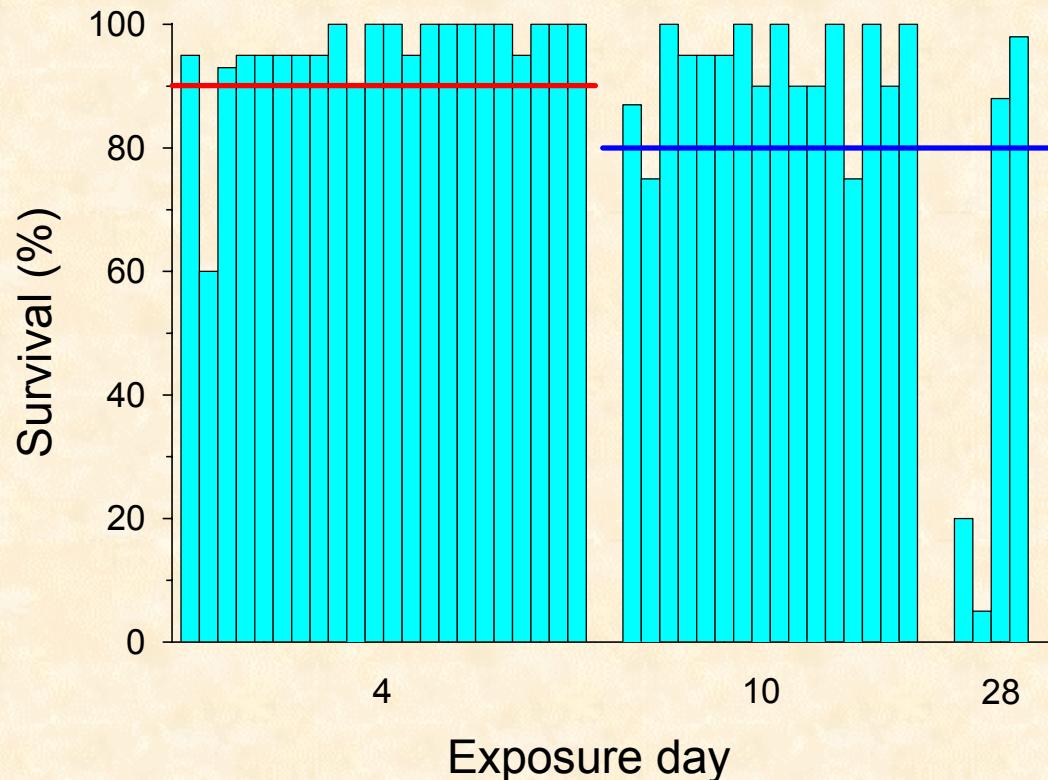
Species	Test water	Survival time (day)		
		>90%	>75%	>50%
Mucket	Well water	4	7	9
	ASTM water	4	5	9
Fatmucket	Well water	5	6	6
	ASTM water	5	6	7

Control survival of glochidia at USGS-Columbia

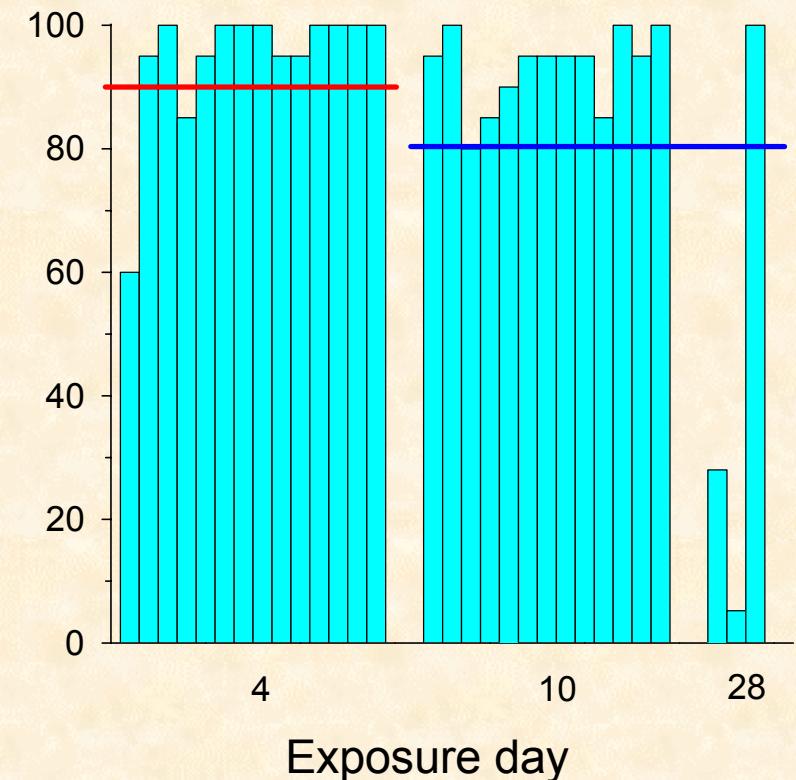


Control survival of juvenile mussels at USGS-Columbia

Copper



Ammonia



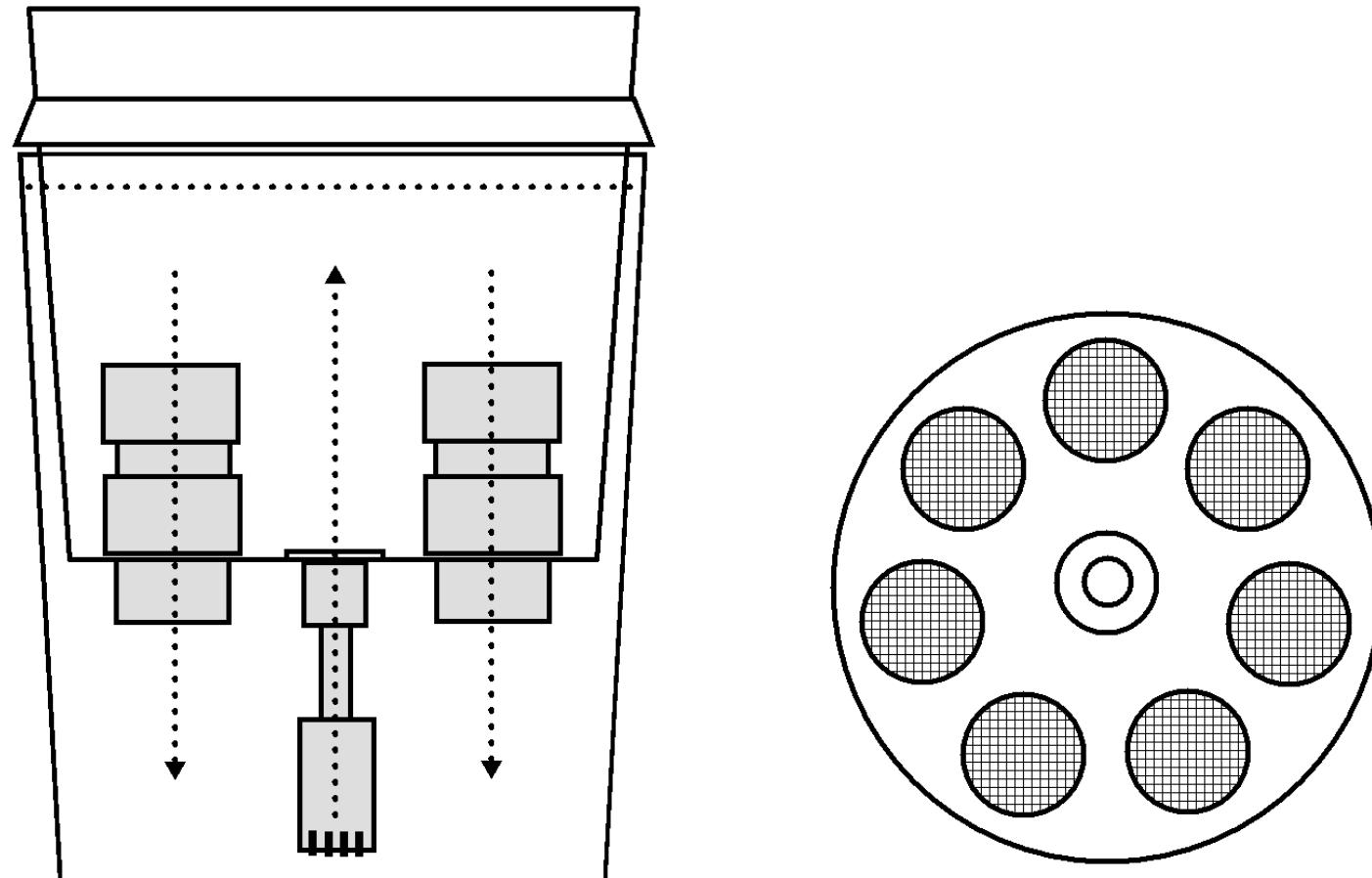
Survival of newly-released juvenile mussels cultured in the laboratory

- Newly-released juvenile mussels obtained from *in vivo* fish host transformation:
 - Critical age: At about 4 weeks transition from pedal feeding to siphoning feeding
- Juveniles fed small algal cells in recirculating or flow-through culturing systems:
 - Large systems (e.g., Neves et al.)
 - Small systems (e.g., Barnhart “Muckets in Buckets”)
 - Flow-through feeding studies (e.g., USGS-Columbia)

Barnhart “Muckets in Buckets”



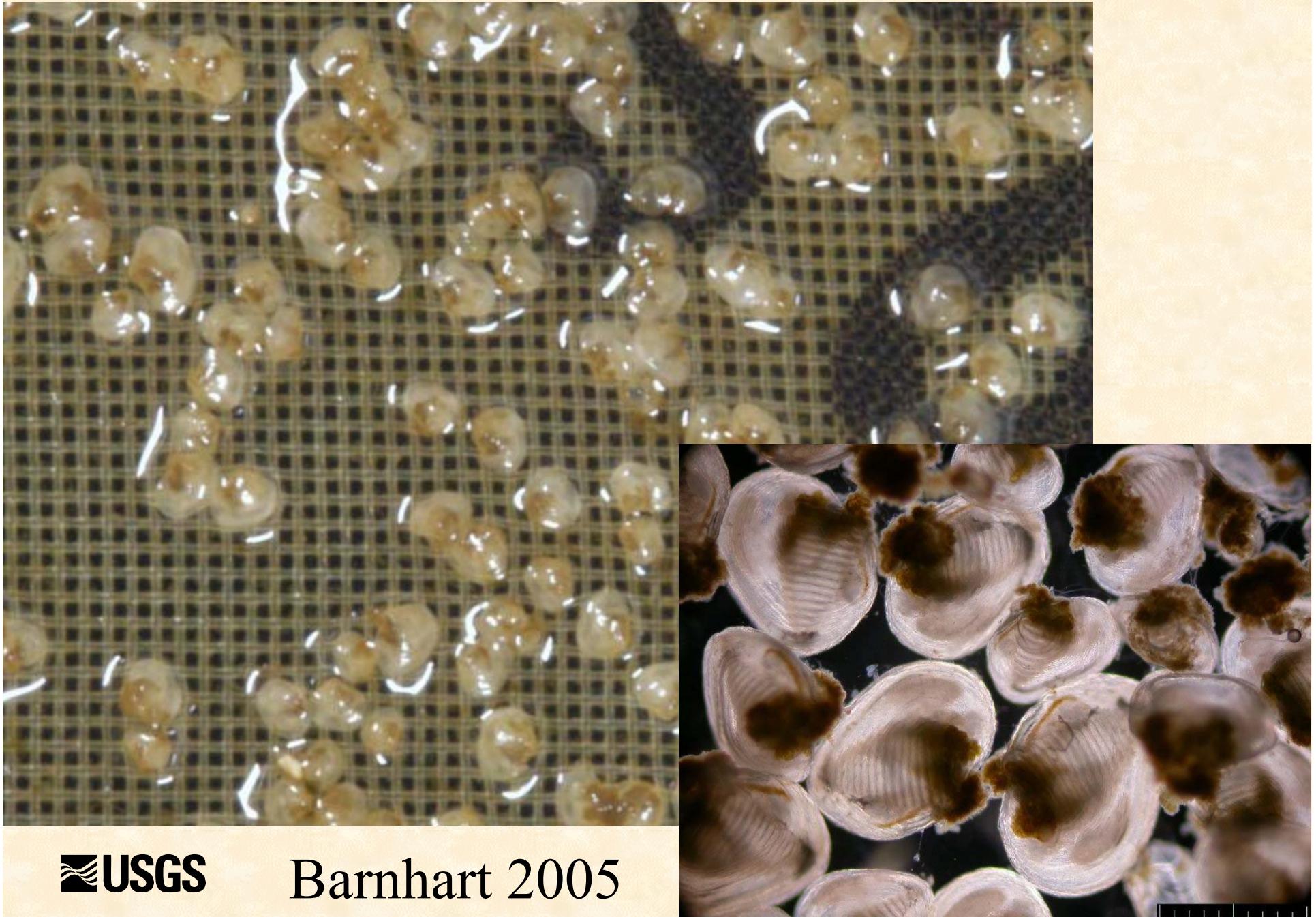
Barnhart “Muckets in Buckets”



Barnhart “Muckets in Buckets”



Barnhart “Muckets in Buckets”

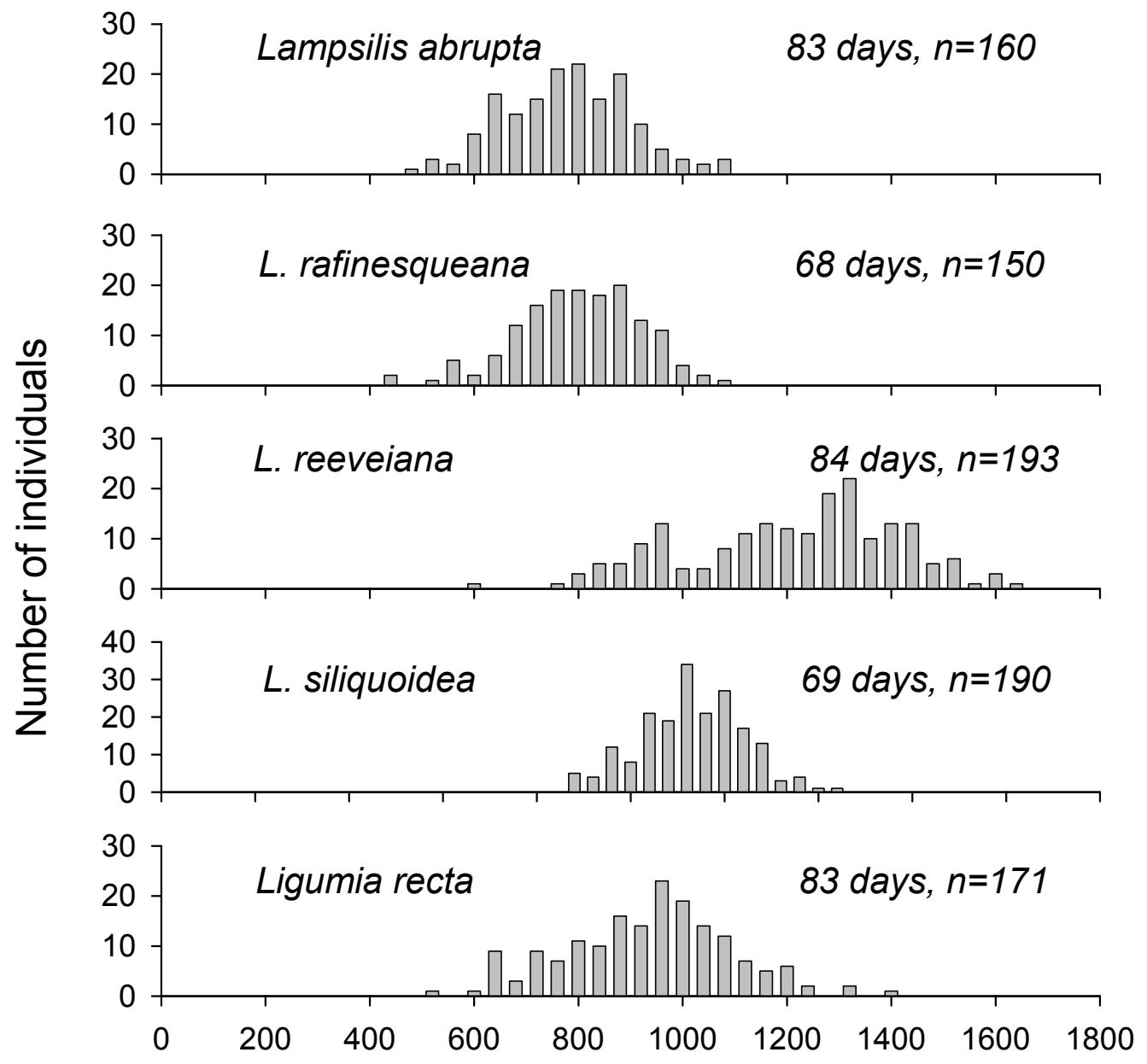


Barnhart 2005

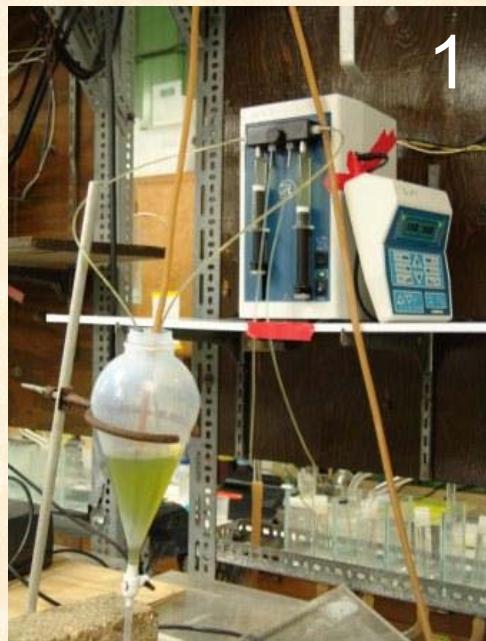
Table 1. Survivorship of 8 species of juvenile unionids after 6 to 10 wk of growth in bucket systems

Species	Age (d)	N total	Percent survival
<i>Epioblasma triquetra</i> (snuffbox)	44	77	24.7
<i>Lampsilis abrupta</i> (pink mucket)	69	1285	84.1
“	69	800	76.0
“	70	1293	79.5
“	70	756	77.6
<i>Lampsilis rafinesqueana</i> (Neosho mucket)	47	690	86.8
“	47	802	87.0
“	47	1262	86.8
<i>Lampsilis reeveiana</i> (broken rays)	68	725	95.2
“	72	296	93.9
<i>Lampsilis siliquoidea</i> (fat mucket)	61	1307	96.1
“	47	1584	95.3
“	47	1684	94.8
“	61	1005	97.4
“	59	1248	97.0
“	48	1373	97.1
“	47	986	94.7
<i>Leptodea leptodon</i> (scaleshell)	84	70	37.1
<i>Ligumia recta</i> (black sandshell)	74	530	75.5
“	69	321	84.4
“	69	293	79.9
“	69	304	87.2
<i>Villosa iris</i> (rainbow mussel)	67	111	72.1

Barnhart 2005



28-d feeding studies with 2-month-old juvenile mussels:



Automatic delivery
of algae to mussels



USGS 2005

28-d survival of 2-month-old juvenile fatmucket fed various foods and amounts

Food	Mean survival (%), n=2		
	Recommend feeding level (RFL)*	2x RFL	3x RFL
<i>Neochloris oleoabundans</i>	75	80	75
<i>Neochloris oleoabundans</i> + sediment	70	55	60
<i>Selenastrum capricornutum</i>	70	50	30
<i>Nannochloropsis oculata</i>	80	45	55
Instant alga (<i>Nannochloropsis</i> sp.)	85	80	65
Instant alga (50%)+Shellfish diet (50%)	90	95	100
No food	35		
Sediment only	25		

*RFL was close to what providers recommended.

28-d survival of 2-month-old juvenile rainbow mussels fed various amounts of CERC instant algae mixture

Groups	N	Survival (%)	
		Mean	SD
Manual feeding: 2 x 2ml	4	100	0
Manual feeding: 2 x 4ml	4	85	24
Auto feeding: 6 x 1ml	4	95	10
Auto feeding: 6 x 2ml	4	98	5
No food	2	50	42

Conclusions

- Test acceptability requirements in E2455-05:
 - 24-h glochidia: 90% survival (must)
 - 96-h juvenile: 90% survival (must)
 - 28-d juvenile: 80% survival (should)
- Frequently met based on:
 - Historic data cited in E2455-05
 - USGS-Columbia data
 - Culture of juvenile mussels in the laboratory